U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE			
PRELIMINARY AMENDMENT		Docket Number **11957/2**	
Application Number To be assigned	Filing Date Herewith	Examiner To the assigned	Art Unit To be assigned
Invention Title **METHOD OF PRODUCING VACCINES FROM PROTEIN SIGNAL OLIGOPEPTIDES**		Inventor(s) MATHIAS RATH	

Assistant Commissioner for Patents
Washington D.C. 20231

SIR:

Please amend the above-referenced patent application as follows:

In the Specification

Page 1, please delete lines 7-10 and insert the following paragraph:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application serial number 09/232,186 filed on January 14, 1999, which is a continuation-in-part of U.S. patent application serial number 08/704,499 filed on August 28, 1996 which is a U.S.C. § 371 of PCT/US95/00575 filed January 12, 1995, which is a continuation of patent application number 08/182,248, filed January 14, 1994, now abandoned, all of which are incorporated herein by reference. - -

Page 25, after line 25, please insert the following:

-- Example No. 6

Cholesterol Measurement in Hep G2 Cells Exposed to MHG CoA Redutase Peptides:

A. Selection of therapeutic oligopeptides for these experiments

i. The peptides were selected according to the method described herein. In brief, 6 oligopeptides corresponding to the hydrophilic amino acid maxima of human hydroxy-methyl-glutaryl-CoA-reductase were selected according to the maximum hydrophilicity

determining algorithm described herein. The following sequences were selected.

I.. N-SQDEVREN-C

II. N-ELSRESREGR-C

III. N-RVLEEEENK-C

IV. N-QKCDSVEE-C

V. N-EETGINRERKVE-C

VI. N-EPEIELPREPRPNEE-C

B. Materials and methods

i. Hep G2 cells were seeded into six-well dishes at 30,0000 cells/well in MEM-supplemented media with 10% FBS. Cells were grown to near confluency and exposed to

I. media alone

II. Mevastatin 5μMol (Cholestrol-lowering Statin drug) and,

III. HMG CoA Reductase peptides at 1, 10 and 100 μMol.

ii. All treatments were carried out in triplicate. After 24 hrs the media was removed and saved.

iii. The cells were washed with 1 ml of PBS and combined with the media. The cells were harvested with 1 ml of 0.1N NaOH.

iv. Samples, both media and cells, were saponified with 0.5 ml 50% KOH, 3 ml ethanol, separately at 80°C for 2 hrs.

v. After cooling, the samples were extracted twice with petroleum ether and washed with NaOH.

vi. The samples were the dried under nitrogen and suspended in 200 μl ethanol.

vii. 50 μl of this suspension was used for determination of cholesterol. Cholesterol was determined in the samples and standards using colorimetric assay (Sigma Kit).

viii. Protein was determined by Lowry's method and the results were expressed as μgm cholesterol/mg protein.

C. Results

i. The following results were obtained:

Peptide	**Treatment**	**Cholesterol Content** Cell	Media	**Total Cholesterol** (μgm/mg protein)	**Cholesterol Lowering in % of Control**
	Control	152.1 ± 39.0	66.5 ± 6.5	218.6 ± 35.0	-
	Mevastatin 5 μM	124.4 ± 4.4	57.5 ± 5.2	182.0 ± 7.0	18.0
1	1μM	92.5 ± 4.7	65.2 ± 4.0	158.7 ± 5.6	28.0
	10μM	88.6 ± 1.6	60.6 ± 0.5	149.1 ± 2.0	30.0
	100 μM	94.0 ± 4.0	61.1 ± 2.4	155.3 ± 6.4	30.0
2	1μM	94.0 ± 8.3	64.3 ± 4.2	158.3 ± 6.3	29.0
	10μM	85.0 ± 8.4	63.7 ± 9.5	148.6 ± 11.8	33.0
	100 μM	86.0 ± 4.7	59.0 ± 6.0	146.9 ± 8.8	34.0
3	1μM	137.3 ± 4.5	64.0 ± 2.7	201.3 ± 3.3	10.0
	10μM	135.0 ± 4.5	65.5 ± 4.7	200.7 ± 9.0	10.0
	100 μM	135.0 7.0	65.5 ± 1.4	200.5 ± 5.4	10.0

<u>Peptide</u>	**<u>Treatment</u>**	**<u>Cholesterol Content</u>** Cell	Media	**<u>Total Cholesterol</u>** (μgm/mg protein)	**<u>Cholesterol Lowering in % of Control</u>**
	Control	151.0± 7.8	50.8 ± 2.5	202.0 ± 5.3	-
	Mevastatin 5μM	125.0 ± 4.6	37.5 ± 3.0	162.6 ± 7.6	19.0
4	1μM	134.7 ± 3.2	50.4 ± 2.5	184.3 ± 5.5	8.0
	10μM	126.1 ± 4.2	49.0 ± 1.0	175.2 ± 3.8	10.0
	100 μM	127.7 ± 9.3	53.8 ± 0.5	181.7 ± 9.3	9.0
5	1μM	140.7 ± 8.6	56.8 ± 1.0	197.0 ± 9.2	1.5
	10μM	149.0 ± 4.4	50.7 ± 1.0	200.0 ± 5.0	0.0
	100 μM	161.7 ± 23.5	48.0 ± 1.6	210.0 ± 25.0	0.0
6	1μM	144.3 ± 7.5	47.4 ± 0.5	192.0 ± 8.0	4.0
	10μM	136.0 ± 12.2	48.0 ± 3.4	184.0 ± 8.4	8.0
	100 μM	132.0 ± 4.4	50.4 ± 1.0	182.4 ± 4.8	9.0

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<u>REMARKS</u>

Applicant respectfully submits that Example Number 6 does not constitute new matter. Rather, Example Number 6 provides experimental data in support of the presently pending claims.

A Final Office Action relating to the U.S. application serial number 09/232,186 was issued on August 8, 2000. Applicant filed a Notice of Appeal on February 8, 2001, therefore an Appeal Brief was due on April 8, 2001. Applicant hereby requests a THREE-MONTH extension time in lieu of an Appeal Brief; i.e., the filing of this continuation.

The Commissioner is hereby authorized to charge payment of the 37 C.F.R. § 1.136(a) extension fee to

the deposit account of **Kenyon & Kenyon**, deposit account number **11-0600**. Applicant is entitled to small entity status, and this and all future fees will be paid at the small entity rate.

Dated: 6/15/01

Respectfully submitted

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